



Study Title: Immunogenicity and safety of heterologous combinations of COVID-19 vaccines available under Emergency Use Authorization in Pakistan: A randomized phase II trial

Short Title: Combination assessment trial of COVID-19 vaccines (COMBAT-COVID)

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2 ABBREVIATIONS

ADEM	Acute Disseminated Encephalomyelitis
AE	Adverse event
AESI	Adverse Event of Special Interest
AKUH	Aga Khan University Hospital
ALT	Alanine Aminotransferase
ARDS	Acute Respiratory Distress Syndrome
AST	Aspartate aminotransferase
BIBP	Beijing Institute of Biological Products
C-19	COVID-19
CBC	Complete Blood Count
CEPI	Coalition for Epidemic Preparedness Innovations
ChAdOx1	Chimpanzee adenovirus 1
CNBG	China National Biotec Group
CoVs	Coronaviruses
CRF	Case Report Form
CTU	Clinical Trial Unit
DRAP	Drug Regulatory Authority of Pakistan
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
ELISA	Enzyme Linked Immunosorbent Assay
ELISPOT	Enzyme-Linked Immune Absorbent Spot
EPI	Expanded Program of Immunization
EUA	Emergency Use Authorization
GCP	Good Clinical Practice
GMR	Geometric Mean Ratio
GTCH	Ghurki Trust Secondary Care Hospital
hACE	Human Angiotensin Converting Enzyme 2
hADV	Human Adenovirus
HIV	Human Immunodeficiency virus
HPB	Heterologous Prime Boost
ICS	Intracellular Cytokine Staining
IHITC	Isolation Hospital and Infection Treatment Centre
IM	Intramuscular
IPD	Invasive Pneumococcal Disease
IPV	Inactivated Polio Vaccine
LGH	Lahore General Hospital
LHR	Lahore
LMIC	Low- and Middle-Income Countries
MERS	Middle East Respiratory Syndrome
NBC	National Bioethics Committee
NIH	National Institute of Health

NRL	Nutrition Research Laboratory
PCR	Polymerase Chain Reaction
PHCs	Primary Healthcare Centers
PHC Global	Precision Health Consultants Global
PI	Principal Investigator
PID	Primary Immune Deficiency
SAE	Serious Adverse Event
SARS-CoV	Severe Acute Respiratory Syndrome Coronavirus
SD	Standard Deviation
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
SS	Sample Size
WHO	World Health Organization
XDR	Extensively Drug-Resistant

3 BACKGROUND

On March 11, 2020 Coronavirus Disease 2019 (COVID-19) was declared a pandemic by World Health Organization (WHO), and within a year of its emergence, this disease with pneumonia like symptoms spread over 220 countries of the world (1). With millions of laboratory-confirmed cases, deaths (WHO), and widespread social and economic disruption, this ever-increasing outbreak has become the biggest challenge of this century (2).

Coronaviruses (CoVs) were first discovered in 1960s (3) and classified as order Nidovirus, family Coronaviridae, subfamily Coronavirinae which is finally divided into α , β , γ and δ genera (4). In last two decades, three CoVs of β genus, SARS-CoV (Severe Acute Respiratory Syndrome Coronavirus), MERS (Middle East Respiratory Syndrome Coronavirus) and SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus-2), have crossed specie barriers and transmitted to humans from animals (5).

All CoVs displays crown-shaped peplomers/spikes on their envelope with large positive-sense single stranded RNA (+ssRNA) (6). One fourth of viral genome is responsible for coding structural proteins, such as the spike (S) glycoprotein, envelope (E), membrane (M) and nucleocapsid (N) proteins. E, M, and N proteins are mainly responsible for virion assembly whilst the S protein is involved in receptor binding that mediates virus entry into host cells during infection (7). The S-protein divides into two subunits, namely, S1 and S2. S1 subunit further subdivided into three domains, particularly A, B, and C. SARS-CoV-2 enters the host cell through direct attachment of domain B (aka receptor binding domain) to the angiotensin converting enzyme-2 (ACE-2) receptor (4).

SARS-CoV-2 became a global threat last year and measures such as social distancing, lockdown and wearing masks were undertaken in countries across the world to prevent the spread of the virus (8). Currently there is no definitive treatment for COVID-19 though Dexamethasone has improved mortality in those with confirmed disease and requiring oxygen support (9).

Cases are rising and many countries are facing second and third waves of infections. Pakistan is also experiencing the third wave of COVID-19 since March 2021 (10). The Drug Regulatory Authority of Pakistan (DRAP) has evaluated and granted emergency use authorization (EUA) to four vaccines against COVID-19. These vaccines were imported from China (Sinopharm/BIBP and CanSino), Russia (Sputnik V, Gamaleya), and United Kingdom (AstraZeneca) (11-13). All these vaccines are approved for use as two-dose regimens except CanSino, which is administered as a single dose (details of the types of the vaccines included in the study and their dosing regimens is detailed in Table 7).

Pakistan formally launched the coronavirus vaccination campaign on February 3rd, 2021 and till March 3rd, 2021, 72,882 individuals have received doses of Sinopharm (14). The first round of vaccination was targeted towards health workers. Pakistan is the world's fifth most-populous country

with a population exceeding 220 million, and programmatic challenges are anticipated in immunising such a large population. It would be advantageous to have a permissive approach of using heterologous prime/boost strategy for vaccination, where the second vaccine is not necessarily the same as the first one. This strategy is already approved and in practice for some vaccines that may be used interchangeably during a vaccination series (from different manufacturers), according to recommendations from the AAP or ACIP, include diphtheria and tetanus toxoids, hepatitis A, hepatitis B, and rabies vaccines (15).

3.1 COVID-19 Situation and Vaccination in Pakistan

Pakistan, world's fifth-most populous country with an estimated 220 million population. The country spreads over an area of 881,913 square kilometres (km²) (16) and continues to face challenges including poverty, illiteracy, unemployment, and poor vaccination coverage (17, 18).

As of September 06, 2021, around 1,182,918 confirmed COVID-19 cases are reported in Pakistan, of which 1,064,319 have recovered and 26,232 deaths (case fatality rate of 2.2%) have been reported (19). Daily over 4000 new confirmed COVID-19 cases are reported from Pakistan. Majority of the cases are reported from Sindh province of Pakistan. In Pakistan, coronavirus vaccinations started on February 3rd, 2021. Chinese government has donated 500,000 doses of Sinopharm vaccine. Furthermore, through the COVAX initiative to ensure the supply of vaccines to all countries, Pakistan has received first batch of 1.2 million doses of AstraZeneca. Pakistan in collaboration with China is also trying to procure 20 million doses of CanSino vaccine, provided that the results of ongoing phase 3 clinical trial show the vaccine to be effective. With the successful completion of Phase 1 of COVID-19 vaccination program in which vaccine is provided to the frontline healthcare workers, current counts report that more than 19,427,419 individuals are fully vaccinated. Though different strategies being employed by Pakistan to acquire enough vaccines for its enormous population of 220 million, considering the costs, far flung areas, and huge population, healthcare officials predict that it is going to be very difficult to immunise a critical mass of individuals which could lead to herd immunity (21, 22).

4 STUDY RATIONALE

The urgency for the development of a COVID-19 vaccine has led to a paradigm shift in process development, where many steps of vaccine development and testing have been performed in parallel to save time. As a result, different types of COVID-19 vaccines emanating from multiple platforms and formulations (23) have progressed rapidly to advanced stages of development. In line with this paradigm shift, the availability of several COVID-19 vaccine candidates could be tested for heterologous prime-boost vaccination strategies (24-27) to elicit higher and broader putative protective immune responses (both antibody and T-cell responses, possibly against circulating

variants of concerns (VOCs)) of longer duration, and for improved safety profiles. It is estimated that over 80% of the adult population have been naturally exposed to the human Adenovirus (hAdV) serotypes which is a component of some viral-vectored COVID-19 vaccine candidates (28). Although not addressed in this study, people previously vaccinated with the standard regimen could be offered a booster immunization with a different vaccine, a different platform.

Circumstances such as vaccine shortages, contraindication to a specific vaccine, changes in product availability, or migration across jurisdictions may necessitate giving vaccines from different manufacturers to the same individual over time. Such data are ascertained and interpreted more readily for diseases with known correlates of protective immunity (e.g., specific serologic markers). For diseases without such surrogate laboratory markers, prelicensure field vaccine efficacy (phase III) trials or post-licensure surveillance generally are required to determine protection (29). Currently, there is a global shortage of COVID-19 vaccines, Pakistan and other Low- and Middle-Income Countries (LMIC) will face a more acute shortage of the vaccines compared to High-Income (HIC) and upper Middle-Income Countries. With the current arrangements full vaccination of all eligible Pakistani population would be challenging. The heterologous prime boost (HPB) approach will help to mitigate the shortage of available COVID-19 vaccines (26, 27). HPB will allow combination of different vaccines in case the same vaccine is not available at the time of boosting (follow-up dose) and longer interval between the two doses could be more pragmatic and practical in real-life settings. Furthermore, the HPB strategy might prove to be more effective against the VOCs of SARS CoV-2. Such a matrix of HPB possibilities has triggered major interest and consideration by manufacturers, funders, and regulators however, data on potential benefits and adverse effects of the HPB matrix of various COVID-19 vaccines is not available. Therefore, we aim to evaluate the safety and immunogenicity of HPB for vaccines approved for use in Pakistan. This exploration meets emergency needs and may assist in the formulation of public health policies.

5 STUDY OBJECTIVES

5.1 Primary Objectives:

1. To determine whether the serum anti-spike IgG concentration against SARS-COV-2 four weeks post boost in COVID seronegative participants immunized with HPB COVID-19 vaccines regimens is non-inferior to that observed following homologous immunization (second dose at Day 70).

5.2 Secondary objectives:

1. To assess the safety and reactogenicity of different combinations of heterologous and homologous COVID-19 vaccines.

2. To characterize the neutralizing antibody response (pseudo-virus neutralization assay) against SARS-CoV-2 (as per schedule of events) in COVID seronegative participants immunized with HPB and homologous COVID-19 vaccines regimens.
3. To assess trend in immunogenicity (anti-spike IgG, anti-nucleocapsid) against SARS-COV-2 as per schedule events in COVID seronegative participants immunized with HPB and homologous CVODI-19 vaccines regimens.
4. To assess safety and immunogenicity of homologous full dose versus fractional dose homologous booster as compared to no booster at 6 months following the second dose of primary series among the participants in homologous arms.

5.3 Exploratory objectives:

1. To characterize SARS-CoV-2 infections (breakthrough infections) and immune responses in participants immunized with COVID-19 vaccines (Whole Genome Sequencing of viral strains).
2. To measure neutralizing antibody response (pseudo-virus neutralization assay) against SARS-CoV-2 VOCs circulating in Pakistan.
3. To assess phenotypic characteristics of CD4 and CD8 T cells by Intracellular Cytokine Staining (ICS) on Immunology cohort
4. To assess the functionality of vaccine-induced antibodies against SARS-CoV2 by performing a panel of systems serology on Immunology cohort.

6 STUDY ENDPOINTS

6.1 Primary Endpoints

1. Anti-spike IgG antibodies by ELISA at Week 14 (4 weeks post booster dose)

6.2 Secondary Endpoints

1. Safety and reactogenicity endpoints are described as below:
 - a. Serious adverse events (SAE) and adverse events of special interest (AESI) throughout the study
 - b. Solicited local and systemic reactions within 7 days post each dose
 - c. Unsolicited reactions within 28 days post each dose
 - d. Medically attended adverse reactions up to 3 months post booster dose
 - e. Changes from baseline to 4 weeks post each dose in biochemical and hematological tests for safety measures
2. Neutralizing antibody response (pseudo-virus neutralization assay) against SARS-CoV-2 at day 0, and weeks 4, 14, 24, 28, 48, 60 and 96 as per schedule of events (*only immunology cohort*) and at baseline and 4 weeks post booster dose in *general cohort*.
3. Immunogenicity of HPB COVID-19 vaccine regimens and homologous immunization, including:

- a. Anti-spike IgG responses measured by ELISA at Day 0, and Weeks 4, 10, 14, 24, 28, 48, 60 and 96 as per schedule of events (*in full cohort*)
- b. Anti-nucleocapsid IgG, responses will be measured at Day 0, and Week 2, 14, 24, 48, 60, and 96 as per schedule of events (*in full cohort*).
- c. ELISpot assays will be performed at Day 0, and week 2, 4, 10, 12, 14, 24, 48, 96 (*immunology cohort*)

6.3 Exploratory Endpoints

1. Neutralizing antibody response (pseudo-virus neutralization assay) against SARS-CoV-2 and anti-spike IgG responses measured by ELISA within 1 week of breakthrough infection among the participants.
2. Sequencing of SARS-CoV-2 viruses of breakthrough infections in vaccine recipients.
3. Neutralizing antibody response (pseudo-virus neutralization assay) against SARS-CoV-2 VOCs circulating in Pakistan.
4. ICS will be performed to assess the phenotypic characteristics of CD4 and CD8 T cells recognizing the antigen by high throughput multicolor flow cytometry. It will measure cytokine secretion after stimulation with Ag and T-cell epitope mapping (optional).
5. Strategic stage-gated Systems Serology analysis will be performed that will not only objectively profile all vaccine induced immune responses but because of this, we will have the opportunity to deprioritize assays and antigens based on rapidly emerging data.

7 METHODOLOGY

7.1 Study Setting

This study will be conducted in Karachi, Lahore, and Islamabad for rapid recruitment and generalizability. Karachi is the capital of Sindh province in Pakistan with a total population of 14.9 million. Karachi is administratively divided into 18 towns. (27). These communities are coastal settlements, with a total area of 8.1 square miles under surveillance.

Volunteers will be identified actively through community mobilization and door to door activity in Ibrahim Hyderi and Ali Akbar Shah Goth extensions of Karachi. Volunteers will be screened for eligibility by the study physicians/research associates during household visits and those who found eligible and consent for participation will be taken to the clinical trial unit of The Aga Khan University for vaccine administration. Volunteers will also be identified actively through community mobilization and door to door activity in sites adjacent to AKUH secondary care Hospitals in Karachi, including The Aga Khan Hospital for Women, Karimabad and Garden. Those who are found eligible and consent for participation will be brought to these two sites for vaccine administration. Other primary health care centers in Karachi are to be decided.

In Islamabad and Lahore volunteers from adjacent areas of Infection treatment center (IHITC) in Islamabad and clinics of Chughtai labs in Lahore will be identified after social mobilization, advertisement and communication and will be brought to these two sites for vaccine administration. Islamabad is the capital city of Pakistan located in the north-eastern part of the country between Rawalpindi and Margalla hills national park to the north. The population of Islamabad is about one million. Isolation hospital and infection treatment centre (IHITC) is 250 bedded hospitals in Islamabad providing free of cost treatment to the people of Islamabad and adjacent cities. IHITC is equipped with central HVAC system, ensuring negative pressure throughout the facility to optimize infection control. Ambulatory services are also available for patients needing transfer to or from IHITC. Additionally, it ensures optimal management for telemedicine and home quarantine patients. Lahore is situated in the north-eastern part of Punjab. The district consists of ten administrative strata, including nine towns and one cantonment area. We will enrol participants at clinics of Chughtai labs. Chughtai labs have a network of vaccination centres, clinics where enrolment procedures, vaccine administration and blood sample collection can be performed, and they are also affiliated with hospitals which can provide care for patients needing in case of any adverse events. Volunteers from adjacent areas after social mobilization, advertisement, and communication with those visiting the vaccination centres/ clinics will be offered invitation for participation in trial. We estimate to enrol 8-10 participants per site /day. There are six trial sites, and we will enrol approximately 120 participants /week and aim to complete the enrolments within approximately 14-16 weeks at this pace.

7.2 Study Design

This will be a randomized, phase II trial, conducted among volunteers aged 18 years and above in Karachi, Lahore, and Islamabad, Pakistan. The study groups are mentioned in Table 1.

7.2.1 Trial duration

The total duration of the trial will be approximately 2 ½ years (14-16 weeks for complete enrolments and two years follow up for each participant following the day of enrolment).

7.2.2 Study Groups

The study will enroll a total of 1680 participants (160 per heterologous group and 240 in each homologous group) which will be divided into 2 cohorts, one for more detailed immunological assessment (immunology cohort, N=225, 25 per group) and one for main immunology endpoints (general cohort N=1455, 135 per heterologous group and 215 per homologous group). We will ensure that the participants of all age groups are represented including less than 40 years and above. The study will include 9 study groups (2:2:2:2:2:3:3:3) with different combinations of COVID-19 vaccines schedule as shown in **Table 1**. This is a pragmatic trial where the interval between the two doses will be kept longer than the conventional recommendations of 21/28 days. Further, we will

include any further homologous vs heterologous groups as more vaccines will receive emergency use approval (EUA) by the drug regulatory authority of Pakistan. The homologous arm will be further sub-divided into three groups to receive either no booster, fractional dose of booster or full dose of booster after six months of second dose.

Table 1: Description of study groups showing interval of vaccine doses and their sample sizes (SS) (SS calculations are provided on page 26)

Group	First Dose	Second Dose	Interval	Booster Dose	SS*
1	BIBP (CNBG, Sinopharm) WIV	CanSinoBIO	70±7 days (10 wks±2)	-	1680 (160 in each heterologous group and 240 in each homologous group)
2	BIBP (CNBG, Sinopharm) WIV	AstraZeneca ChAdOx			
3	CanSinoBIO	BIBP (CNBG, Sinopharm) WIV			
4	CanSinoBIO	AstraZeneca ChAdOx			
5	AstraZeneca ChAdOx	BIBP (CNBG, Sinopharm) WIV			
6	AstraZeneca ChAdOx	CanSinoBIO			
7(ref)**	BIBP (CNBG, Sinopharm) WIV	BIBP (CNBG, Sinopharm) WIV		6 months post second dose	
8(ref)**	CanSinoBIO	CanSinoBIO			
9(ref)**	AstraZeneca ChAdOx	AstraZeneca ChAdOx			
*SS: Sample Size; a subset of each group (25 per group) will be allocated to the immunology sub cohort					
** 240 participants each will be enrolled in the reference/homologous groups					

7.3 Study Participants and Eligibility Criteria

7.3.1 Trial participants

Adult male and female volunteers aged 18 years and above and volunteers with well controlled mild or moderate comorbidities will be enrolled to participate in trial.

7.3.2 Inclusion criteria

- Participant is willing and able to give written informed consent for participation in the trial.
- Male or Female aged 18 years or above and in good health as determined by a trial clinician. Participants may have well controlled mild-moderate comorbidity.
- In the Investigator's opinion, is able and willing to comply with all trial requirements.
- Residing in the study areas.

7.3.3 *Exclusion criteria*

The participant may not enter in the trial if ANY of the following apply:

- Pregnant women or those who are planning to conceive within next 70 days.
- Women who are breast feeding
- Already received any COVID-19 vaccine or any other vaccine likely to impact on interpretation of the trial data (e.g., Adenovirus vectored vaccines).
- Administration of immunoglobulins and/or any blood products within the three months preceding the planned administration of the vaccines.
- Any confirmed or suspected immunosuppressive or immunodeficient state; asplenia; recurrent severe infections and use of immunosuppressant medication within the past 6 months, except topical steroids or short-term oral steroids (course lasting ≤ 14 days)
- History of allergic disease or reactions likely to be exacerbated by any component of study vaccines (e.g., hypersensitivity to the active substance of the COVID-19 vaccines included in the study groups)
- Any history of anaphylaxis.
- Current diagnosis of or treatment for cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ)
- Bleeding disorder (e.g., factor deficiency, coagulopathy, or platelet disorder), or prior history of thrombotic events and/or significant bleeding or bruising following IM injections or venipuncture.
- Continuous use of anticoagulants, such as coumarins and related anticoagulants (i.e., warfarin)
- Any other significant disease, disorder or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data.
- Severe and/or uncontrolled cardiovascular disease, respiratory disease, gastrointestinal disease, liver disease, renal disease, endocrine disorder, and neurological illness (mild/moderate well controlled comorbidities are allowed)
- History of active or previous auto-immune neurological disorders (e.g., multiple sclerosis, Guillain-Barre syndrome, transverse myelitis). Bell's palsy will not be an exclusion criterion.
- History of laboratory confirmed COVID-19 within 6 months prior to enrolment (history of SARS-CoV-2 detection by PCR or antibody to SARS-CoV-2).
- Scheduled elective surgery during the trial.
- Participants enrolled in any other research trial.
- Participants planning to migrate out of the study area within 2 years of the study.

7.3.4 Temporary exclusion criteria

If the volunteer has any of the following, they will not be enrolled that day.

- Acute respiratory illness (moderate or severe illness with or without fever)
- Fever (temperature greater than 38°C)

They may be considered for enrolment later in the trial if they recover in sufficient time.

7.4 Participants' recruitment and screening

Potential participants will be invited to participate in the trial by door-to-door home visitation in the selected communities. There will also be publicizing via flyers at the enrolment sites i.e., vaccination centres and clinics. A study information leaflet will be provided to the volunteers so that they can understand the trial and procedure of participation. A written version and verbal explanation of the study information leaflet will be presented to the participant, detailing:

- The exact nature of the study
- What it will involve for the participant
- The implications and constraints of the protocol
- The known side effects and any risks involved in taking part.
- The sample handling protocol.

The Study information leaflet will be made available to the participant for an appropriate amount of time (where possible this will be a minimum of 24 hours) prior to consent being obtained.

7.4.1 Screening

Once volunteers express an interest in joining the trial after going through the information leaflet, they will be directed to the designated sites for screening and eligibility assessment by the study staff including study physician. If volunteer is found eligible for participation, informed consent will be collected.

7.4.2 Informed consent

The participant will personally sign and date the latest approved version of the informed consent form. A witness or interpreter will be arranged for those who cannot read or understand our language. Consent form will be available in Urdu and English languages. Participants will have the opportunity to individually question an appropriately trained and delegated researcher before signing consent.

The following general principles will also be emphasised:

- Participation in the study is entirely voluntary.
- Refusal to participate involves no penalty or loss of medical benefits.
- The participant may withdraw from the study at any time.
- The participant is free to ask questions at any time to allow him or her to understand the purpose of the study and the procedures involved.

- Those participants will not be sure whether they have received an approved COVID-19 vaccine schedule. This may have implications for any travel or other activities that may require individuals to be considered ‘fully immunised’.
- Participants, like the general population, will not be exempt from following the contemporaneous government COVID-19 guidance to minimise viral transmission.
- Samples taken as part of the study may be sent outside of Pakistan to the laboratories for testing. Samples will be de-identified. Volunteers will be asked if they consent to indefinite storage of any leftover samples for use in other ethically approved research, this will be optional.

The person who obtained the consent must be suitably qualified and experienced and have been authorised to do so by the Chief/Principal Investigator and listed on the delegation log. A copy of the signed informed consent will be given to the participant. The original signed form will be retained at the research study office.

7.5 Randomisation

Computer generated randomisation list will be prepared and participants will be individually randomized using the block randomization technique. A block size of 21 will be used to randomize nine heterologous/homologous groups. The initial 25 participants in each group will be included in the immunology cohort. All participants enrolled in each of the three homologous groups will be randomized for booster dose at 6 months following the second dose of vaccine administration to receive either full dose of the homologous booster, or fractional dose intradermal booster or no booster. Since each homologous group has 240 participants, about 80 participants in each group will receive full dose booster, 80 will receive fractional dose and 80 will receive no booster.

7.6 Details of Study Follow-ups/Visits

The study visits and procedures will be undertaken by study team. The procedures to be included in each visit are documented in the schedule of events (**Table 2**). Each visit is assigned a time-point and a window period, within which the visit will be conducted. If a participant cannot attend a visit, where possible, this will be re-arranged to an in-person visit within the time window. A phone call will be made to collect as much relevant information as possible if the participant is unable to attend the visit in person, because of quarantine or self-isolation restrictions, and the participant will be out of window period if the visit is further postponed.

Table 2: Schedule of Events

Variables	V1	V2	V3	V4	V5	V6	V7	V8	Additional visit only for homologous arms		V9	V10	V11	C-19 (COVID-19) pathway§
Study timeline (Days)	D0	D14	D28	D70	D84	D98	D168	D196	D238	D266	D336	D420	D672	D0-D672
Study timeline (Weeks)	W0	W2 (2 wks. post first dose)	W4	W10	W12 (2 wks. post 2 nd dose)	W14	W24 (6 months post first dose)	W28	W34	W38 (4wks post booster dose)	W48 (1 yr. post first dose)	W60	W96 (2 yrs. post first dose)	ASAP after the COVID- 19 test report
Window period		±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	
Informed consent	X													
Medical history	X													
Interim medical history			X	X		X		X			X	X	X	X
Physical examination (including vital signs etc. as required)	X		(X)	(X)		(X)		(X)			(X)	X	(X)	(X)
COVID-19 vaccination	X			X					X					
Home Visit		X	X	(X)	X	X	X	X	(X)	X	X	X	X	X
Safety Bloods (*Biochemistry & **Hematology) BoC	X		X			X				X				X
Phone calls for AE	Weekly till 14 days after each dose in addition to Monthly phone calls													
Physician Teleclinic Visit														X
Pseudovirus neutralization assay(BoC)	X		X			X	X	X		X	X	X	X	X
Anti-spike IgG (BoC)	X		X	X		X	X	X	X	X	X	X	X	X
Anti-nucleocapsid IgG (BoC)	X		X			X	X			X	X	X	X	X
SARS-CoV-2 viral swab (Nasal swab)														X
Systems Serology (IC)	X	X	X	X	X	X	X		X	X	X		X	X
Blood sample for ICS & ELISpot (IC)	X	X	X	X	X	X	X		X	X	X		X	X

V: Visit, D: Day X: Additional measurements only for immunology cohort.

§ Whole genome sequencing for all breakthrough cases and targeted sequencing for regional VOCs. BoC: Both cohorts IC: Immunology cohort

*Biochemistry: Na, K, Urea, Creatinine, Bilirubin, ALT, ALT Phosphatase, Albumin, and AST

**Hematology: CBC

Visit 1 (D0): Baseline assessment, eligibility check, enrolment, and vaccination visit

7.7 Eligibility Screening:

At the initial visit participants will be assessed at the study site, by study staff including study physician for eligibility to participate. A structured questionnaire (CRF 1) will be used to evaluate the volunteer for all eligibility criteria. If any of the exclusion criteria will be found volunteer will be declared as not eligible. If some temporary illness or condition is found, the volunteer will be declared as temporary not eligible and will be called for another screening session in due time, if feasible according to study timelines.

7.7.1 Informed Consent Process:

If volunteer is found eligible, he/she will be offered participation in the trial and informed consent will be administered. Complete informed consent document will be read and explained to each potential participant despite the fact that study information leaflet had already been handed over to them. A trained study team member will conduct the consent taking process and will get the form signed, or thumb impression taken with date by participant and witness, or interpreter as required. One signed copy of consent form will be handed over to the participant and original form will be kept in study office.

7.8 Randomization:

Once consent is obtained, enrolment procedure will be initiated. Enrolment will be done on the day of vaccine first dose administration. Participants will be randomized in one of the study groups and will be enrolled in immunology or general cohort. Participants in homologous group will be further randomized for booster doses as well.

7.9 Blood sample collection:

Blood sample will be taken as per laboratory specifications to assess immunogenicity and serological assays including baseline serology to confirm seronegative against SARS-CoV2 at the time of first dose administration.

7.10 First dose of vaccine:

Volunteers will be considered enrolled to the trial at the point of vaccination. All vaccines will be administered intramuscularly according to specific SOPs. The participant will stay in the trial site for observation for at least 15 minutes, for any immediate adverse events.

7.11 Interview and CRF-2 filling:

CRF 2 will be filled on the day of enrolment which will record information on their demographic and basic clinical data, including co-morbidities and concomitant medications and their weight height will also be measured.

7.12 Phone Calls/ Home Visits for Recording AE And Safety Concerns:

All participants will be given an emergency 24-hour telephone number to contact the on-call study physician if needed. Participants will be instructed on how to self-assess the severity of the adverse events (AEs). Paper tape will be provided to each participant to measure the erythema or swelling (if any) following vaccination. Participants will be contacted via phone calls/home visit weekly until D14 post vaccination and monthly thereafter to record AE or any serious medical illnesses or unplanned hospital/visits and adverse events of special interest. These follow-ups will collect information on the timing and severity of the following solicited AEs (**Table 3**). Adverse events of special interest (AESI) will be collected and recorded in the AE reporting form in electronic data capturing system throughout the duration of this study. These should also be reported as SAEs if they fulfil the definition criteria for SAEs (definitions of adverse events provided in **Table 4**). All AESI's not already reported as SAEs should be included in the reports to the IDMC.

7.13 Subsequent Visits:

There will be home visits, in addition to 2 or 3 clinic visits for vaccine doses. During each call and visit, CRF will be filled and collected information will be recorded.

At each visit:

- Participant identification will be verified by vaccination card/ trail registration card.
- Brief medical history will be taken.
- All married women of childbearing age will be enquired about pregnancy.
- Physical examination will be performed (if indicated).
- Well-being, adverse reactions, or side-effects will be assessed and managed (if applicable).
- Information on symptoms compatible with COVID-19, testing for and results of testing for the infection will be collected.
- Blood sample will be collected as per laboratory specifications to assess AEs, and immunogenicity assays.
- Vaccine dose will be administered (if applicable)

Prior to administering the second dose and booster dose of the vaccine, any newly available and relevant safety data will be reviewed from animal studies or clinical trials of coronavirus vaccines included in this study being tested elsewhere and discussed with the Independent Data Monitoring Committee (IDMC), as necessary. While there will be no planned safety pause, a review of safety data will be conducted after the initial 50 - 60 participants have received a second dose at the day

70±7 days post first time-point. This will assess safety in the first 48 hours after immunisation. Further details about the IDMC, its mandate, frequency of meetings, and its charter is provided in section 9.2 on page 38.

7.14 Adverse Events:

Table 3: Solicited Adverse Events to Record Post Vaccination

Local solicited AEs	Pain, Tenderness, Redness, Warmth, Itch, Swelling/ Induration
Systemic solicited AEs	Fever, Feverishness, Chills, Joint pains, Muscle pains, Fatigue, Headache, Malaise, Nausea, Vomiting, Diarrhoea

Table 4: Adverse Event Definitions

AE	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
AESI	Adverse events identified as being of relevance to the IMP's. These will also be reported as an SAE, if meeting SAE criteria (e.g., hospitalisation)
SAE	<p>SAE is any untoward medical occurrence that:</p> <ul style="list-style-type: none"> • Results in death • Is life-threatening • Requires inpatient hospitalization or prolongation of existing hospitalization • Results in persistent or significant disability/incapacity • Consists of a congenital anomaly or birth defect* <p>Other 'important medical events' may also be considered a SAE when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.</p> <p>NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.</p>

Table 5: Adverse events of special interest

Immunologic	Anaphylaxis	
Neurological	Isolated anosmia/ageusia*	Meningoencephalitis
	Guillain-Barre Syndrome	Peripheral facial nerve palsy
	Acute disseminated	Generalized convulsion
	encephalomyelitis (ADEM)	Myelitis

	Aseptic meningitis	
Hematological	Thrombosis** Stroke Thrombocytopenia*** Eosinophilia****	Coagulation disorder (includes coagulopathy, thrombosis, thromboembolism, internal/external bleed, and stroke) Thrombosis with Thrombocytopenia Syndrome (TTS)
Cardiac	Acute cardiovascular injury (includes myocarditis, pericarditis, arrhythmias, heart failure, infarction)	Multisystem Inflammatory Syndrome in Adults (MIS-A)
Dermatological	Chilblain-like lesions Single organ cutaneous vasculitis	Erythema multiforme Alopecia
Gastrointestinal	Acute liver injury †† †	Appendicitis
Respiratory	ARDS††	
Renal	Acute kidney injury	
Other	COVID-19 disease†	SARS-CoV2 positivity on a validated test
<p>*In the absence of COVID-19</p> <p>** Excluding superficial thrombophlebitis (including line-associated)</p> <p>*** G3 or above</p> <p>**** This will be used as a marker of skewed Th2 responses and will be routinely monitored in participants attending the COVID-19 Pathway and follow-up visits. Only G2 and above.</p> <p>† In particular, any occurrence of suspected vaccine associated enhanced disease (VAED) as defined by most recent Brighton Collaboration Case Definition</p> <p>†† In the absence of an infective etiology (including COVID-19)</p> <p>†† † As defined in Hy's Law (see Error! Reference source not found.)</p>		

7.15 Participants under quarantine

Given the evolving epidemiological situation both globally and in Pakistan, when a participant will be unable to attend any of their scheduled or unscheduled visits, a telephone consultation will be arranged to obtain core study data where possible. Participants should not attend for in-person visits if they are in their period of self-isolation/quarantine – the exception to this is the COVID-19 Pathway.

If blood sample collection is involved, then the trained study staff in complete PPE as per recommendation by WHO will visit the home.

7.16 Participants with Confirmed SARS-CoV-2 Infection (COVID-19 Pathway)

Participants will be counselled at enrolment that if they receive a positive SARS-CoV-2 test (e.g., an antigen detection or nucleic acid amplification test, for example, via test and trace or occupational health services) they should contact the trial team on receipt of the positive result. Participants will be reminded of this with a phone call which will commence after the first vaccine dose.

Once the participant has conveyed his/her result to the study team, and the study team confirm an appropriate test has been used (verbal discussion with participant as to how testing was obtained, confirmatory documentation will not be sought), a physician tele-clinic appointment will be arranged within 7 days of reporting by the study staff to assess the participants' clinical severity at the relevant study site and a sample collection at home will be arranged. At this visit nasal swab for storage and subsequent viral isolation and whole genome sequencing (WGS) and targeted sequencing for VOCs will be collected and a blood sample for safety assessment (if clinically indicated). Vital signs (if possible) and other clinical data will be recorded. The window period for performing this visit is mentioned in Table 6.

Table 6. COVID-19 Pathway for the enrolled participants during the study period.

Variables	Margin
Reporting of participants to Study staff about COVID-19 status	As soon as possible after the COVID-test report
Physician Teleclinic	Within 7 days of reporting to study staff
Nasal Swab for COVID-19 PCR, WGS and targeted sequencing and/or blood sampling (if clinically indicated)	Within 7 days of Teleclinic

Participants will be screened for severity of disease during their visit and referred to the nearby tertiary care hospital/government facility if there will be any shortness of breath or chest tightness. Participants will be counselled to follow national/local guidelines/SOPs for isolation/infection prevention measures.

7.16.1 Whole genome and targeted genome sequencing for regional VOCs

As RNA viruses are known to mutate at higher rates compare to DNA viruses, SARS-CoV-2 has also been mutated significantly over time compared to the first reported Wuhan strain and has resulted in the generation of different variants. Some of these variants because of higher transmissibility or infection rate and escape from neutralizing antibodies could become dominant variants and are known

as VOCs. Genomic surveillance played a pivotal role in identification of these variants to understand the evolution and genomic diversity of SARS-CoV-2 and is helping us to design better diagnostic tests and vaccines against the virus. Therefore, in the proposed study we are aiming to perform targeted whole genome sequencing (WGS) of SARS-CoV-2 isolates using MinION platform (Oxford Nanopore Technologies) at AKUH for the identification of VOCs of SARS-CoV-2 for all breakthrough infections during this trial.

7.17 Sample Handling

7.17.1 Blood sampling, transportation, and storage

For subsequent visits, appointment will be taken through telephone at least 24-48 hrs prior and the team will visit the participant at home to collect the blood sample. A trained staff will collect blood sample according to requirement of study cohort using aseptic technique and will transfer these samples to respective sites in temperature-controlled environment. Blood samples will be processed, transport and stored according to the SOPs of respective laboratories for required tests.

Safety monitoring tests for AEs will be performed at clinical lab AKUH Karachi and AKUH stat laboratories Lahore and Islamabad. PBMCs from whole blood will be separated at AKUH Karachi for ELISpot and ICS. ELISpot will be performed at AKUH with the assistance of our collaborator, Oxford University who have fully validated protocol to perform ELISpot. ICS will be performed at Oxford university. NIH Laboratory Islamabad will also separate PBMCs for ELISpot and ICS. At all sites PBMCs will be stored in liquid nitrogen till further use. The Infectious Disease Research Laboratory (IDRL) at AKUH Karachi will archive and ship all samples to respective labs for particular assays.

Humoral immunogenicity will be assessed for antibodies to SARS-CoV-2-Spike and nucleocapsid antigens by ELISA and pseudovirus neutralization assay. Samples will be processed for Anti-Spike IgG, Anti-Nucleocapsid IgG and Pseudo-neutralizing antibody responses at AKUH laboratory. AKUH laboratory is accredited by the College of American Pathologists (CAP). A subset will be sent to the CEPI laboratory network for analysis which will also be serve as External Quality Assessment (EQA). Ragon Institute, Harvard Medical School, Boston MA, USA, will provide training to researchers from AKUH, Pakistan to perform system serology assay to assess functional antibodies, using the Luminex based technology which will allow rapid profiling of the overall levels of antibody isotypes, subclasses, Fc-receptor binding antibodies (to 6 different Fc-receptors), complement binding antibodies, and Fc-glycan changes (using lectins) and to profile the humoral immune response across the vaccine Spike antigen (RBD, NTD, S1, S2) as well as RBD and full Spike VOCs (alpha, beta, delta, gamma, etc). ELISA for Anti-spike IgG will also be performed at Nutrition Research Laboratory (NRL) AKUH. At AKUH blood samples will be processed in biosafety level three (BSL-

3) facility at Juma Research Laboratory as per COVID-19 biosafety guidelines. All time points, volume of blood collected at each time point and designated assays have mentioned in Appendix A and Appendix B of protocol.

Subjects will be informed that there may be leftover samples of their blood (after all testing for this study is completed), and that such samples may be stored indefinitely for possible future research (exploratory immunology), including genotypic testing of genetic polymorphisms potentially relevant to vaccine immunogenicity. Subjects will be able to decide if they will permit such future use of any leftover samples. With the participants' informed consent, any leftover cells and plasma will be frozen indefinitely for future analysis of COVID-19 and other coronaviruses related diseases or vaccine-related responses. If the subjects elect not to permit this, all those participants' leftover samples will be discarded at the end of the trial.

7.17.2 Nasal swabs

Nasal swabs will be collected from study participants with breakthrough infection and subjected to RT-PCR assay for the detection of SARS-CoV-2 viral RNA. Remaining RNA samples from PCR confirmed-cases will be stored at -80°C until subject to WGS. WGS will be performed for the identification of VOCs of SARS-CoV-2 for all breakthrough infections during this trial.

7.18 Early Discontinuation/Withdrawal of Participants

In accordance with the principles of the current revision of the Declaration of Helsinki and any other applicable regulations, a participant has the right to withdraw from the study at any time and for any reason and is not obliged to give his or her reasons for doing so. The investigator may withdraw the participant at any time in the interests of the participants' health and well-being. In addition, the participant may withdraw/be withdrawn for any of the following reasons:

- Administrative decision by the Investigator
- Ineligibility (either arising during the study or retrospectively, having been overlooked at screening).
- Significant protocol deviation
- Participant non-compliance with study requirements
- An AE, which requires discontinuation of the study involvement or results in inability to continue to comply with study procedures

The reason for withdrawal will be recorded in the CRF. If withdrawal is due to an AE, appropriate follow-up visits or medical care will be arranged, with the agreement of the volunteer, until the AE has resolved, stabilised or a non-trial related causality has been assigned. The IDMC chair may recommend withdrawal of participants.

If a participant withdraws from the study, storage of samples will continue unless the participant specifically requests otherwise. Any data collected before their withdrawal will still be used in the analysis for trial integrity; if the participant requests this could be de-identified following the end of the study.

7.19 Contra-Indications to Receipt of Second/Booster Dose of Vaccine

The following AEs associated with any vaccine, identified on or before the day of vaccination constitute absolute contraindications to further administration of a study vaccine to the participant in question. If any of these events occur during the study, the subject will not be eligible to receive a second or booster dose and will be followed up by the clinical team or their physician until resolution or stabilisation of the event:

- Anaphylactic reaction following administration of vaccine
- Any vaccine-related SAE, AESI, particularly any thrombotic event
- Any AE that in the opinion of the Investigator may affect the safety of the participant or the interpretation of the study results

Participants who develop COVID-19 symptoms and have a positive SARS-CoV-2 antigen test/ PCR after the first vaccination can only receive a booster dose after a minimum 4-week interval from their first positive test, provided their symptoms have significantly improved. The decision to proceed with booster vaccinations in those cases will be at clinical discretion of the investigators. For participants who are asymptomatic and have a positive SARS-CoV-2 test, a minimum of 2 weeks from first test positivity will be required before boosting provided they remain asymptomatic. Married women of childbearing age who report to be pregnant before the administration of the booster dose will not be offered the heterologous vaccine booster. They will be referred to an obstetrician for pregnancy follow-up and to the nearest government vaccination team for the administration of the homologous vaccine dose. The data of these women will be excluded from the final analysis. However, we do not expect losing more than a couple of women who might get pregnant after the first dose (despite of counselling and clear assurance from their side to avoid pregnancy at the time of enrolment).

7.20 Definition of End of Trial

The end of the trial is the last participant last visit.

7.21 Trial Intervention

Below are the details of the types of the vaccines included in the study and their dosing regimens.

Table 7: Details of Trial Intervention

Vaccine	Type	Vector	Vial	Volume of Per Dose (ml)	Site of administration	No. of Doses	Time Interval	Route	Storage Temp.	Recommended Age group (years)	Efficacy	References
AstraZeneca-University of Oxford (UK)	Viral Vector	Chimpanzee Adenovirus encoding the SARS-CoV-2 Spike glycoprotein (ChAdOx1-S) ChAdOx1-S (recombinant)	Multidose contains 5 ml	0.5 ml	Deltoid muscle (upper arm)	10	4-12 weeks apart	IM	2-8°C	> 18	Single dose: 76% Two doses (4 weeks apart): 54.9% Two doses (12 weeks apart): 82.4%	(34, 35)
CanSinoBio (China)	Viral Vector	Ad5-nCoV	Prefilled syringe	0.5 ml	Deltoid muscle (upper arm)	1	NA	IM	2-8°C	> 18	Single dose: 65.7% (moderate disease), 91% (severe disease)	(36)
Sinopharm/BIBP (China)	Whole Inactivated Virus	NA	Prefilled syringe	0.5 ml	Deltoid muscle (upper arm)	1	3 weeks apart	IM	2-8°C	>18	Two doses: 79-86%	(37)

7.22 Storage of Study Vaccines

Vaccines will be stored in accordance with manufacturers' recommendations at the NIH, Islamabad, Pakistan and shipped in batches to the study sites in Karachi, and Lahore. In Lahore, the vaccines will be stored at Chughtai lab facility according to manufacturer's recommended temperatures and doses for daily usage will be received from the cold storage facility in Coleman. Similarly, At AKUH vaccines will be stored at clinical trial unit (CTU) and will be dispatched to respective sites. All movements of the study vaccines will be documented in accordance with existing standard operating procedure (SOP). Vaccine accountability, storage, shipment, and handling will be in accordance with relevant SOPs and forms.

7.23 Compliance with Trial Treatment

All vaccinations will be administered by the research team and recorded in the CRF. The study vaccine will be at no time in the possession of the participant. There will be reminders and facilitation for clinic visit for vaccination to maximize compliance.

7.23.1 Accountability of the trial treatment

The vaccine supplies will be supervised at all times by study teams. Vaccine counts will occur to ensure that no vaccines are missing. Dispensation and return of study vaccines will be recorded in the Study Vaccine Accountability Log according to the standards.

7.23.2 Concomitant medication

Concomitant medications taken at enrolment will be recorded, as will new medications have taken within the 28 days after each immunisation. Subsequently only new medications taken in response to a medically attended adverse event up until 3 months post second and booster dose will be recorded.

7.23.3 Post-trial treatment

If any heterologous boost regimen is not found to be immunogenic, decisions regarding the need for a booster dose, the nature of the booster dose and mode of delivery (e.g., national program vs. study site) will be made in consultation with the IDMC and study management group.

7.23.4 Other treatments

Participants will be advised that they may take paracetamol prophylactically after vaccine administration. This will be from the participants own supplies rather than supplied by the study team.

7.23.5 Other interventions

There are no additional investigations other than those specified in this protocol.

7.24 Sample Size

The participants will be divided into two cohorts one for detailed immunological assessments (immunology cohort) and one for main immunology endpoints (General cohort). We choose the

sample size of 160 participants per heterologous group and 240 per homologous group (total **N=1680**) who will be boosted at D70, including 25 participants per group in the immunology cohort and 135 per heterologous and 215 per homologous group of the general cohort.

The below sample size calculation is based on the primary endpoints of anti-spike IgG and neutralizing antibody responses to live SARS-CoV-2. The current available data from COMCOV at University of Oxford suggest the standard deviation (SD) of anti-spike IgG (Nexelis) at 4 weeks post boost is around 0.3-0.4 at log10 scale among participants received different vaccine schedules (4 weeks first boost interval), and the SD of neutralizing antibody response to live SARS-CoV-2 (normalised MNA50) is 0.4 at log10 scale among 194 participants in COV001 and COV002 (prime-boost interval of 4-12 weeks).

The assumptions for sample size calculation are:

1. The non-inferiority margin for geometric mean ratio (GMR) is 0.63 between heterologous and homologous boost groups.
2. The standard deviation is 0.4 on log10 scale.
3. The true GMR is 1.

Based on the above assumptions, the study will need to recruit 100 participants who are seronegative at baseline in each group to achieve 90% of power to detect the non-inferiority of immunogenicity of heterologous group compared to homologous group at one-sided 1.25% significance level to preserve a family-wise type 1 error rate of one-sided 2.5% since there will be two formal comparisons within each of the group administered with 3 different vaccines. After accounting for ~25% seropositivity rate at baseline and ~15% attrition rate, the study will need to recruit 160 participants per group. This means that total sample size will be $N=160 \times 9 = 1440$. In order to be able to assess the immune response of any potential heterologous booster (3rd dose) introduced among the homologous groups in future, we will enrol more than the expected number of participants in the homologous group (instead of 160 per homologous group, 240 will be enrolled), thereby increasing the required SS to **N=1680**. With the expanded size in the homologous arms, the power for the primary endpoint analyses will be >90%.

The immunogenicity cohort will be used for exploratory analyses to generate hypothesis, and thus no formal sample size calculation was carried out for this cohort. The sample size of 25 per group was therefore chosen based on practical constraints. This means we will have around 20 seronegative participants in each group for the analysis.

7.25 Plan of Statistical Analysis

The primary analysis of this study will be a non-inferiority comparison between heterologous and homologous boost schedules, i.e., Groups 1/2 vs. 7, 3/4 vs. 8, and 5/6 vs. 9 as defined in **Table 1**. All the primary analyses will be done among the seronegative population at baseline. For the primary endpoint analyses, the GMC of anti-spike IgG and neutralizing antibody responses will be compared between different heterologous boost groups and their corresponding homologous group at Day 98 (4 weeks post booster dose). We will claim heterologous boost group is non-inferior to homologous boost group if the lower limit of two-sided 97.5% CI of GMR between heterologous boost group and their corresponding homologous group lies above 0.63. The sensitivity analysis will be done to estimate the adjusted GMR with two-sided 97.5% CI after controlling for the randomisation design factor of study site. The GMC will be transferred using logarithmic transformations (base 10) to render a normal distribution. Trend in the levels of GMC over the period in each group will be plotted and comparison between heterologous groups and their corresponding homologous group at several timepoints will be performed. The primary analysis will be carried out when the primary endpoints of D98 anti-spike IgG and/or neutralizing antibody response data become available. The two primary endpoints analyses will be considered separately. The success of one endpoint will not be contingent on the success of the other. Therefore, either or both the primary endpoints could be successful for the study. No multiplicity adjustment will be made for the two primary endpoints as they are likely to be highly correlated. The formal test of non-inferiority for two different heterologous groups compared to homologous group will be conducted with significance level of one-sided 1.25% using Bonferroni approach. There will no adjustment for multiple testing for secondary analyses and a two-sided 0.05 will be considered statistically significant. All the other secondary immunogenicity endpoints analysis in terms of further immunogenicity characteristics or different timepoints will be descriptively summarized.

The analysis population for the primary endpoints will be on the per-protocol basis as the intent-to-treat analysis is no longer a conservative estimate for non-inferiority comparisons. Sensitivity analyses for primary endpoints on the modified intent-to-treat basis will be also conducted.

For safety analysis, we will descriptively summarize the number of event and number of participants and proportion of participants who experienced SAE, AESI, solicited and unsolicited AE, medically attended adverse reactions by vaccine group. The safety analysis population will be the participants who received at least one dose of the study vaccines.

An interim analysis will be carried out when the serology data at 4 weeks post second dose become available for the first 300 participants at baseline. If the seropositivity rate is higher than 25%, we will re-evaluate the study power and increase the sample size if needed.

Further interim analyses for primary and secondary endpoints will be carried out when the data becomes available to support policy making.

A fully detailed statistical analysis plan will be prepared and will be signed off by the PI prior to database lock for conducting any data analyses.

8 DATA MANAGEMENT

The PI will be responsible for all data that accrues from the study.

8.1 Missing Data

The level and pattern of the missing data in the baseline variables and outcomes will be reported. The potential causes of any missing data will be investigated and documented as far as possible. Any missing data will be dealt with using methods appropriate to the conjectured missing mechanism and level of missing.

8.2 Access to data & data protection

Direct access will be granted to authorised representatives from the funder, host institution and the regulatory authorities to permit trial-related monitoring, audits, and inspections. The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorised third party, without prior written approval of the funder.

8.3 Data collection and recording

All study data including participant diary will be recorded directly into an electronic CRF using electronic data capture (EDC) system (e.g., REDCap or any other validated EDC system) or onto a paper source document for later entry into EDC if direct entry is not available. This includes sociodemographic, safety, and laboratory and outcome data. All documents will be stored safely and securely in confidential conditions. The eCRF data will be stored at data management unit server located at AKUH, Pakistan.

The participants will be identified by a unique trial specific number and code in any database. The name and any other identifying detail will NOT be included in any trial data electronic file, except for the electronic data records for AEs, for which consent will be obtained to store the participant's name and phone number for quality control purposes. The diaries (with identifiers) will only be visible/accessible to the investigator(s) and relevant study staff (not funder) and is to serve the purpose of being able to query data from the subject if needed. The sponsor has well defined SOPs which describe how only deidentified data will be shared with the funder.

The data backups will be maintained according to the set standards of the data management unit of the Department of Paediatrics and Child Health, AKUH, Karachi. Access to the study's database will be restricted to members of the study team by username and password.

8.4 Record keeping

The Investigators will maintain appropriate medical and research records for this trial, in compliance with GCP and regulatory and institutional requirements for the protection of confidentiality of volunteers. The PI and Co-Is will have access to records. The Investigators will permit authorised representatives of the funder and Host institution, as well as ethical and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress.

Identifiable information such as contact details will be stored for a minimum of 5 years from the end of the study. This includes storage of consent forms. Storage of these data will be reviewed every 5 years and files will be confidentially destroyed if storage is no longer required. Considerations at the time of this review will include the value of retaining this information for participant safety (e.g., to inform participants of unexpected safety signals emerging from post-licensing surveillance), as a resource for the participants (e.g., if they wish to check which vaccines they have received in the study) and any regulatory requirements. De-identified research data maybe be stored indefinitely. If volunteers consent to be contacted for future research, a record of this consent will be recorded, retained, and stored securely and separately from the research data. If volunteers consent to have their samples stored and used in future research, information about their consent form will be retained and stored securely as per Biobanking procedures and SOP.

8.5 Source data and case report forms (CRFs)

All protocol-required information will be collected in CRFs designed by the Investigator. All source documents will be filed in the participant file. Source documents are original documents, data, and records from which the participant CRF data are obtained. For this study, these will include, but are not limited to, volunteer consent form, blood results, laboratory records, diaries, medical records. In most cases, CRF entries will be considered source data as the CRF is the site of the original recording (i.e., there is no other written or electronic record of data). In this study this will include, but is not limited to medical history, medication records, vital signs, physical examination records, adverse event data and details of vaccinations. All source data and participant files will be stored securely.

8.6 Data quality

Data collection tools will undergo appropriate validation to ensure that data are collected accurately and completely. Datasets provided for analysis will be subject to quality control processes to ensure analysed data is a true reflection of the source data. Trial data will be managed in compliance with local data management SOPs. If additional, study specific processes are required; an approved Data Management Plan will be implemented.

8.7 Quality Assurance Procedures

8.7.1 Risk assessment

The trial will be conducted in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures. A risk assessment and monitoring plan will be prepared before the study opens and will be reviewed as necessary over the course of the trial to reflect significant changes to the protocol or outcomes of monitoring activities.

8.7.2 Monitoring

Monitoring will be performed according to Good Clinical Practice (GCP) guidelines by an external monitor Precision Health Consultants (PHC) Global. PHC Global, registered in Pakistan since 2012 is an organization lead by public health experts and professionals with extensive experience and in depth understanding of research conduct and public policy formulation based on internationally accepted frameworks in health and education. PHC Global functions under a board of directors following robust standards of financial, and institutional policies. In addition, PHC Global's in house team comprises of social scientists, anthropologists, economists, and biostatisticians with their branches available in Karachi, Lahore, and Rawalpindi in Pakistan as well as the USA, Canada, and Australia and support multi-disciplinary projects. In this project PHC global will monitor and verify that the clinical trial is conducted as per the SOPs, and data are generated, documented, and reported in compliance with the protocol, GCP and the applicable regulatory requirements. The investigator sites will provide direct access to all trial related source data/documents and reports for the purpose of monitoring and auditing by the funder or the Host institution and inspection by local and regulatory authorities. In the presence of travel restrictions having a CRO from within the country is in the best interest of the study.

9 TRIAL COMMITTEES

9.1 Joint steering committee

A Joint Steering Committee (JSC) will be formed to oversee the study and advise the Study Management Committee on key issues of study conduct, including, but not limited to, study pauses due to safety concerns on the advice of the independent data monitoring committee (IDMC). The JSC will be comprised of independent (external) technical experts with relevant research experience from CEPI & consortium members (investigators) and nominated focal person from CEPI. The study management committee will be comprised of only study investigators and research managers. The JSC will be referred for deliberation and expert guidance related to the technical issues regarding the study protocol, amendments, design etc. They will also be informed regularly about the progress of the study and challenges being faced. The study management committee is internal body which will meet frequently (every week in the initial 2 months, followed by fortnightly meetings & need based) to discuss the study progress, enrolment, challenges, and issues. They will take decisions regarding

day-to-day issues however, high level decision related to the change in study protocol will be referred to the JSC.

9.2 Independent data monitoring committee (IDMC)

An independent data monitoring committee (IDMC) will be convened. All collaborators/consortium members will be non-voting observers along with a SPEAC Meta-DSMB expert (also non-voting observer). The IDMC will evaluate frequency of adverse events, safety data as specified in the IDMC charter (to be developed later). The IDMC will make recommendations concerning the conduct, continuation, or modification of the study for safety reasons to the JSC. The IDMC will review SAEs or AESIs deemed possibly, probably, or definitively related to study interventions. The IDMC will be notified within 24 hours of the Investigators' being aware of their occurrence. The IDMC can recommend placing the study on hold if deemed necessary following a study intervention-related SAE. IDMC will conduct the review of safety data after the initial 50 - 60 participants have received a second dose at the day 70 ± 7 days post first time-point. Further, the IDMC will meet quarterly to evaluate data for any safety signals, SAEs and any other issues.

10 PROTOCOL DEVIATIONS

A trial related deviation is a departure from the ethically approved trial protocol or other trial document or process (e.g., consent process or IMP administration) or from Good Clinical Practice (GCP) or any applicable regulatory requirements. Necessary trainings will be provided to the study staff and logs will be maintained to avoid deviations however, in case of any deviation from the protocol, it will be documented in a protocol deviation form and filed in the trial master file. Some of the major deviations are defined below:

- Schedule of events: for each event or visit, there is a specified timepoint and a window period during which the visit should be performed. Any deviation from that window will be considered as protocol deviation.
- Study vaccines: for each group the first, second and booster dose of the vaccine is pre-specified as per protocol. Once the participants are randomized to the assigned group, the vaccine as per protocol in the given group should be administered. If the given vaccine is not administered or first and second vaccines (in heterologous arms) are exchanged, this will be considered as protocol deviation.
- missing any exclusion criteria during screening and finding after enrolment.
- Randomization error
- Missing any sample

11 ETHICAL AND REGULATORY CONSIDERATIONS

The Investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki and in accordance with Good Clinical Practice. Ethical approval will be obtained from the national bioethics committee of Pakistan (NBC), AKUH Ethical Review Committee, IRB approvals from IVI and Oxford University and approval from Drug Regulatory Authority of Pakistan (DRAP). The submissions to the respective IRBs/ECs and regulatory authorities will be made in parallel and we expect 6-8 weeks of time for receiving the complete set of approvals.

12 TRIAL REGISTRATION

Prior to the recruitment of the first participant, the trial will have been registered on a publicly accessible database and the trial information will be kept up to date during the trial, and the PI or their delegate will upload results to the trial registry within 12 months of the completion of the trial follow-ups.

13 EXPENSES AND BENEFITS

Volunteers will be compensated with 2000 Pakistani rupees per visit for their time and travel expenses. If the patient suffered from any adverse event causally related to the given vaccine, the cost of treatment including laboratory investigations will be provided from the study.

14 EXPERIENCE IN CONDUCTING VACCINE RESEARCH (38-46)

The AKUH has well-developed infrastructure for conducting clinical trials. A Clinical Trials Unit supports drug/vaccination importation and management, GCP training and compliance. The study team have recently successfully conducted complex polio vaccine trials including; 1) Poliovirus type 2 challenge study, a community based randomized controlled trial, 2) Assessment of intradermal adaptors in administration of fractional dose IPV among children, 3) Polio endgame strategies: Polio Virus Type 2 Challenge study in Pakistan: five groups randomized control trial, 4) Intradermal Administration of Fractional Dose of Inactivated Poliovirus Vaccine Using Intradermal Adaptors vs. BCG syringe: Community Based Randomized Control Trial in Pakistan, 5) Intradermal Administration of Fractional Dose of Inactivated Poliovirus Vaccine (fIPV) Using Tropis® Intradermal Needle Free Injection System, Karachi Pakistan, 6) Comparison of IPV and fractional dose IPV in routine immunization: A community based Randomized Control Trial in Pakistan, 7) Pilot Surveillance of poliovirus excretion among persons with primary immune deficiency in Pakistan & Assessment of Sensitivity of Jeffery Modell Foundation Signs for screening of Suspected PID Children. In addition, the team has also successfully completed impact evaluation of pneumococcal conjugate vaccine against invasive pneumococcal disease (IPD) in Sindh, impact evaluation of typhoid conjugate vaccine in an outbreak setting of XDR typhoid in Hyderabad and Lyari town Karachi, and

a five-year longitudinal cohort study evaluating sero-efficacy of typhoid conjugate vaccine among children in Hyderabad is currently in progress.

15 POTENTIAL CHALLENGES:

- Study delays/timelines may be affected in the event of lockdowns due to COVID-19.
- Vaccine hesitancy in general population will be a challenge.
- Regulatory approvals from DRAP (Drug Regulatory Authority of Pakistan) may affect the study timelines.
- Unexpected disruptions to the supply of the Project Vaccines, recalls due to safety issues or withdrawals of the Project Vaccines can be a risk for the trial

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	V1	V2	V3	V4	V5	V6	V7	Additional visit only for homologous arms		V8	V9	V10	COVID-19 Pathway
Timeli ne	D0	D14	D28	D70	D98	D168	D196	D238	D266	D336	D420	D672	D0-D672
	W0	W2 (2wks post first dose)	W4	W10	W14 (4wks post 2 nd dose)	W24 (6months post first dose)	W28	W34	W38 (4wks post booster dose)	W48 (1 yr. post first dose)	W60	W96 (2 yrs. post first dose)	W0-W96
Safety Tests	AKUH/NIH/LHR Biochemistry Hematology	-	AKUH/NIH/LHR Biochemistry Hematology	-	AKUH/NIH/LHR Biochemistry Hematology		-	-	AKUH/NIH/LHR Biochemistry Hematology	-	-	-	AKUH/NIH/LHR Biochemistry Hematology
Safety Blood	1xGel top (~5ml) 1xEDTA (~3ml)	-	1xGel top (~4ml) 1xEDTA (~3ml)	-	1xGel top (~5ml) 1xEDTA (~3ml)		-	-	1xGel top (~5ml) 1xEDTA (~3ml)	-	-	-	1xGel top (~5ml) 1xEDTA (~3ml)
Prima ry Endpo int (PE)	-	-	-	-	AKUH/ CEPI Anti-spike IgG Neut. Abs								
Secon dary Endpo int (SE)	AKUH/ CEPI Anti-spike IgG Neut. Abs Anti-Nucleocapsid IgG		AKUH/ CEPI Anti-spike IgG Anti-Nucleocapsid IgG	AKUH Anti-spike IgG	AKUH CEPI Anti-Nucleocapsid IgG	AKUH Anti-spike IgG Nucleocap sid IgG	AKUH Anti-spike IgG	AKUH Anti-spike IgG	AKUH Anti-spike IgG Nucleocapsi d IgG Neut. Abs	AKUH Anti-spike IgG Nucleoca psid IgG	AKU H Anti-spike IgG Anti-Nucleo capsid IgG	AKUH Anti-spike IgG Anti-Nucleocap sid IgG	AKUH/ CEPI Anti-spike IgG Neut. Abs Anti-Nucleocapsid IgG
Blood for PE + SE	2xEDTA (~3ml each)		2xEDTA (~3ml each)	2xEDTA (~3ml each)	2xEDTA (~3ml each)	2xEDTA (~3ml each)	2xEDTA (~3ml each)	2xEDTA (~3ml each)	2xEDTA (~3ml each)	2xEDTA (~3ml each)	2xEDT A (~3ml each)	2xEDTA (~3ml each)	2xEDTA (~3ml each)

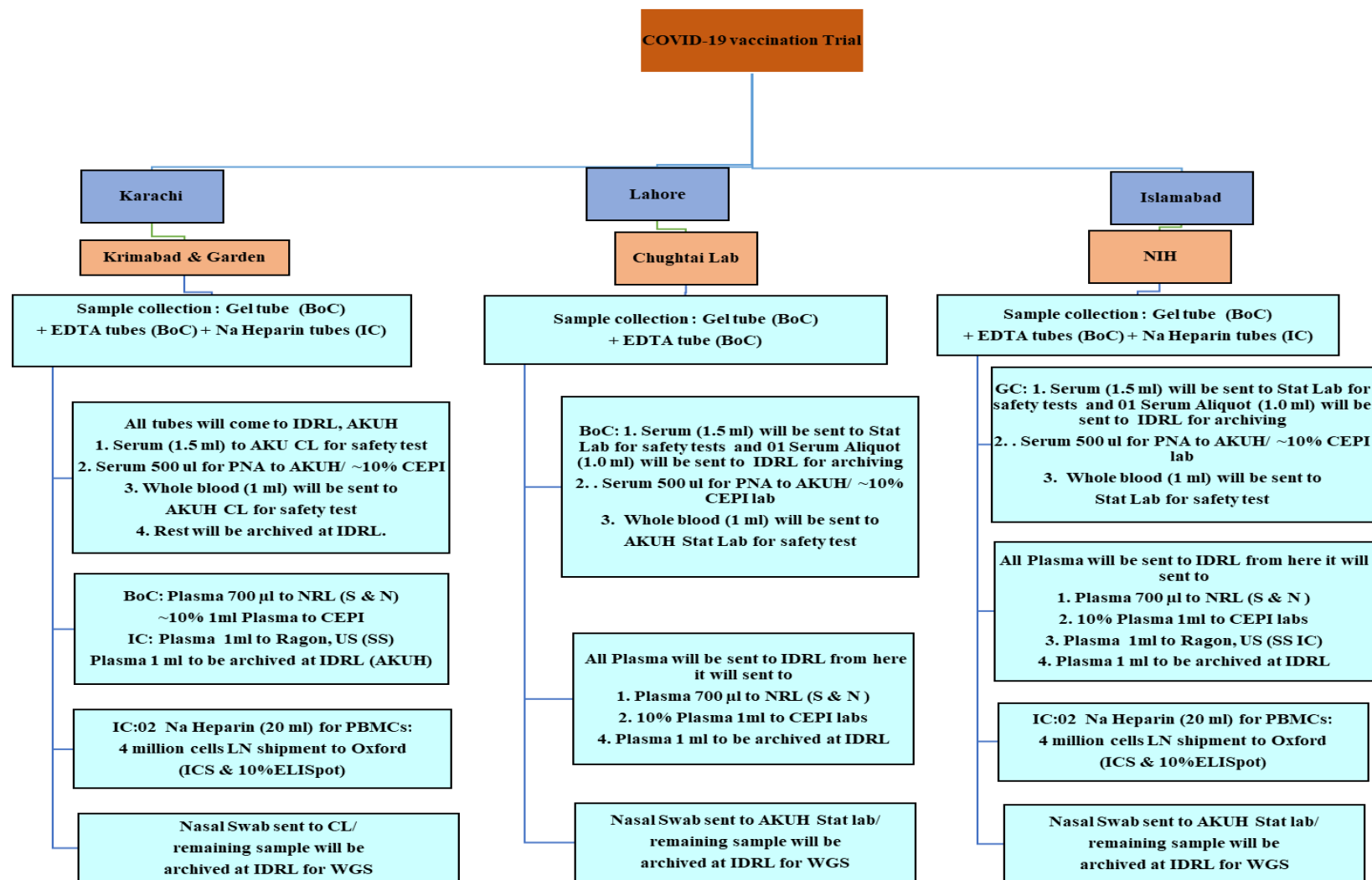
Total Blood/ visit	~14ml (1xGel top ~5ml) (3xEDTA ~3ml each)	~3ml (1xEDT A)	~13ml (1xGel top ~4ml) (3xEDTA ~3ml each)	6ml (2xEDT A ~3ml each))	~14ml (1xGel top ~5ml) (3xEDTA ~3ml each)	6ml (2xEDTA ~3ml each)	~6ml (2xEDTA ~3ml each)	6ml (2xEDT A ~3ml each))	~14ml (1xGel top ~5ml) (3xEDTA ~3ml each)	~6ml (2xEDT A ~3ml each)	~6ml (2xED TA ~3ml each)	~6ml (2xEDTA ~3ml each)	~14ml (1xGel top ~5ml) (3xEDTA ~3ml each)
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Immunology Cohort

	V1	V2	V3	V4	V5	V6	V7	V8	Additional visit only for homologous arms		V9	V10	V11	COVID-19 Pathway
Timeline	D0	D14	D28	D70	D84	D98	D168	D196	D238	D266	D336	D420	D672	D0-D672
	W0	W2 (2wks post first dose)	W4	W10	W12 (2wks post boost)	W14 (4wks post 2 nd dose)	W24 (6months post first dose)	W28	W34	W38 (4wks post booster dose)	W48 (1 yr. post first dose)	W60	W96 (2 yrs. post first dose)	
Safety Tests	AKUH/NI H/LHR Biochemis try Hematolo gy	-	AKUH/N IH/LHR Biochemi stry Hematolo gy	-	-	AKUH/NI H/LHR Biochemis try Hematolo gy	-	-	-	-	-	-	-	AKUH/NIH/L HR Biochemistry Hematology
Safety Blood	1xGel top (~5ml) 1xEDTA (~3ml)	-	1xGel top (~5ml) 1xEDT A (~3ml)	-	-	1xGel top (~5ml) 1xEDTA (~3ml)	-	-	-	1xGel top (~5ml) 1xEDTA (~3ml)	-	-	-	1xGel top (~5ml) 1xEDTA (~3ml)
Primary Endpoint (PE)						AKUH/ CEPI Anti-spike IgG Neut. Abs								
Secondary Endpoint (SE)	AKUH/ CEPI Anti-spike IgG Neut. Abs Anti- Nucleocap sid IgG Oxford/ AKUH ELISpot ICS	Oxford/ AKUH ELISpot ICS	AKUH/ CEPI Anti- spike IgG Nucleoca psid IgG Neut. Abs Oxford/ AKUH ELISpott ICS	AKUH Anti- spike IgG Oxford U/ AKUH ELISpot ICS	Oxford/ AKUH ELISpot ICS	AKUH/ CEPI Anti- Nucleocap sid IgG Oxford/ AKUH ELISpot ICS	AKUH Anti-spike IgG Neut. Abs Anti- Nucleocap sid IgG Oxford/ AKUH ELISpot ICS	AKU H Anti- spike IgG Neut. Abs	AKUH Anti-spike IgG Oxford/ AKUH ELISpot ICS	AKUH Anti-spike IgG Anti- Nucleocap sid IgG Neut. Abs Oxford/ AKUH ELISpot ICS	AKUH Anti-spike IgG Neut. Abs Anti- Nucleocap sid IgG Oxford/ AKUH ELISpot ICS	AKUH Anti- spike IgG Neut. Abs Anti- Nucleoca psid IgG	AKUH Anti-spike IgG Neut. Abs Anti- Nucleocapsi d IgG Oxford/ AKUH ELISpot ICS	AKUH/ CEPI Anti-spike IgG Neut. Abs Anti- Nucleocapsid IgG Oxford/ AKUH ELISpot ICS

Explorator y Endpoint (EE)	Harvard/ AKUH Systems Serology	Harvard/ AKUH Systems Serology	Harvard/ AKUH Systems Serology	Harvard/ AKUH Systems Serology	Harvard/ AKUH Systems Serology	Harvard/ AKUH Systems Serology	Harvard/ AKUH Systems Serology		Harvard/ AKUH Systems Serology	Harvard/ AKUH Systems Serology	Harvard/ AKUH Systems Serology		Harvard/ AKUH Systems Serology	Harvard/ AKUH Systems Serology
Blood for PE +SE+EE	2xEDTA (~3ml each) 2x Na Heparin (~10ml each)	2xEDT A (~3ml each) 2x Na Heparin (~10ml each)	2xEDTA (~3ml each) 2x Na Heparin (~10ml each)	2xEDTA (~3ml each) 2x Na Heparin (~10ml each)	1xEDTA (~3ml) 2x Na Heparin (~10ml each)	2xEDTA (~3ml each) 2x Na Heparin (~10ml each)	2xEDTA (~3ml each) 2x Na Heparin (~10ml each)	2xEDT A (~3ml each)	2xEDTA (~3ml each) 2x Na Heparin (~10ml each)	2xEDTA (~3ml each) 2x Na Heparin (~10ml each)	2xEDTA (~3ml each) 2x Na Heparin (~10ml each)	1xGel top (~3ml) 2xEDTA (~3ml each)	2xEDTA (~3ml each) 2x Na Heparin (~10ml each)	2xEDTA (~3ml each) 2x Na Heparin (~10ml each)
Total Blood/visit	~34ml (1xGel top ~5ml) (3xEDTA ~3ml each) (2x Na Heparin ~10ml each)	~26ml (2xEDT A ~3ml each) (2x Na Heparin ~10ml each)	~34ml (1xGel top ~5ml) (3xEDTA ~3ml each) (2x Na Heparin ~10ml each)	~26ml (2xEDTA ~3ml each) (2x Na Heparin ~10ml each)	~23ml 1xEDTA (~3ml) 2x Na Heparin (~10ml each)	~34ml (1xGel top ~5ml) (3xEDTA ~3ml each) (2x Na Heparin ~10ml each)	~26ml (2xEDTA ~3ml each) (2x Na Heparin ~10ml each)	~6ml (2xEDT A)	~26ml (2xEDTA ~3ml each) (2x Na Heparin ~10ml each)	~34ml (1xGel top ~5ml) (3xEDTA ~3ml each) (2x Na Heparin ~10ml each)	~26ml (2xEDTA ~3ml each) (2x Na Heparin ~10ml each)	~9ml (1xGel top ~3ml) (2xEDT A ~3ml each)	~26ml (2xEDTA ~3ml each) (2x Na Heparin ~10ml each)	~34ml (1xGel top ~5ml) (3xEDTA ~3ml each) (2x Na Heparin ~10ml each)

17.2 Appendix B: Flow of Sample Processing



BoC: Both cohorts GC: General cohort IC: Immunology cohort CL: clinical lab PNA: Pseudo neutralization assay S: Anti-spike IgG N: Anti-nucleocapsid IgG SS: System serology